

## Determination of total active oligosaccharide content of cream formulation of *Triticum vulgare* L. extract by ion chromatography

Yeter Arten , Şafak Şanlı  and Nurhan Metan 

Abdi Ibrahim İlaç Sanayi Quality Control Analytical Method Validation and Technology Transfer Laboratory, 34519 Istanbul, Türkiye

(Received May 25, 2025; Revised June 24, 2025; Accepted June 25, 2025)

**Abstract:** *Triticum vulgare* L. extract is a natural wound healer widely used in pharmaceutical production cream formulations due to its ability to accelerate tissue repair. This study reports the method validation of an ion chromatography method developed to determine the total amount of active oligosaccharide in a finished product formulation. A Thermo Dionex Carbopac PA1 (4 mm x 250 mm) column connected to a Thermo Dionex Carbopac PA1 Guard (4 mm x 50 mm) guard column was used as the analytical column in the High-Performance Anion Exchange Chromatography with Emphasized Amperometric Detector method. A total run time of the method was applied as 85 minutes and 95% to 5% gradient of Mobile Phase A (Sodium Hydroxide) and Mobile Phase B (Sodium Acetate Trihydrate-Sodium Hydroxide) solutions were used with 0.5 mL/min flow rate. The developed method was validated in terms of specificity, precision, linearity and range, accuracy and recovery parameters according to the ICH Q2 (R1) guideline. The linear regression equation of the method was determined as  $y=6204.20x-737.0873$  ( $r:0.999$ ), while % recovery was obtained as  $103.4 \pm 3.6$ .

**Keywords:** *Triticum vulgare* L.; ion chromatography; method validation; cream; extract. © 2025 ACG Publications. All rights reserved.

### 1. Introduction

*Triticum vulgare* L. is a type of wheat that was developed in 1899 by Mark Alfred Carleton for adaptation trials to countries with different climate conditions from America and some have been made [1]. The extract obtained from the germ part of the seed is a clear, colorless or pale-yellow liquid and is rich in vitamin E, folic acid, fatty acids and fatty alcohols [2]. For this reason, it has a wide range of use in the production of pharmaceutical products in the cosmetics and pharmaceutical industries [2]. Oligosaccharides obtained from *Triticum vulgare* L. extract contribute to the acceleration of tissue repair processes in skin lesions. Due to these properties, it is widely used in traditional medicine. Studies show that this extract reduces inflammation by suppressing the production of pro-inflammatory mediators such as interleukin-6 (IL-6), injection of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), prostaglandin E2 (PGE2) [3]. In vitro studies on microglial cells have shown that *Triticum vulgare* L. extract may also have protective effects against nervous system inflammation [3]. It protects the skin from allergies, bacteria and irritants by forming a thin film layer that acts as a barrier on the skin surface through its rich natural secondary metabolites. It has been reported that it helps prevent skin problems such as acne, eczema, dryness and itching. It has also been reported that it supports the protection of the lipid barrier by filling the gaps

\* Corresponding author: E-Mail: [nurhan.metan@abdiibrahim.com.tr](mailto:nurhan.metan@abdiibrahim.com.tr)

between dead cells. In traditional Iranian medicine, wheat germ oil is known to be used for dermo-tonic effects and skin beauty, facial freckles, moisturizing and repairing small pores. *Triticum vulgare* L. extract is known to be a rich source of oligosaccharide as well as vitamin E, folic acid and fatty acid components [1].

Extensive research has been conducted on various types of oligosaccharides. They differ in the nature of their monomeric sugars, which is how they are named. They have different sources of origin and provide different benefits to consumers. The most popular oligosaccharides are fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose-derived galactooligosaccharides (LDGOS), xylooligosaccharides (XOS), arabinooligosaccharides (AOS) and algae-derived marine oligosaccharides (ADMO). Other oligosaccharides that occur naturally include pectin-derived acidic oligosaccharides (pAOS), maltooligosaccharides (MOS), cyclodextrins (CD) and human milk oligosaccharides (HMO), which have specific acknowledged benefit [4,5]. These compounds promote a healthy balance of intestinal microflora and reduce the risk of gastrointestinal infections. The beneficial physiological functions of functional oligosaccharides in humans can be summarised as follows: 1- they do not stimulate an increase in blood glucose or insulin secretion, as they dissolve in the gut to form a viscous gel which lowers glucose absorption; 2- they supply a small amount of energy (approximately 0–3 kcal/g); 3- they are non-cariogenic; 4- they improve the intestinal environment and alter the intestinal microbiota, resulting in an increase in salutary bacteria due to the acidic intestinal environment; 5- they alleviate and suppress diarrhoea and its symptoms; and 6- they stimulate the intestinal absorption of minerals such as calcium, magnesium and iron [6]. Furthermore, consuming functional oligosaccharides reduces the risk of diseases such as cardiovascular disease, colon cancer and obesity [7]. Health products supported by extensive scientific research continue to stimulate great interest and demonstrate potential for future growth. Functional oligosaccharides are used as stabilizers, bulking agents, immunostimulating agents or prebiotic compounds in feeds, pharmaceuticals and cosmetics [6]. The synthesis of oligosaccharides is crucial in the field of medicine [8]. However, obtaining homogeneous samples during glycosylation is challenging. Historically, chemical synthesis has been used as an exploratory research method to prepare homogeneous oligosaccharides [9].

Oligosaccharides are polymeric compounds formed by the combination of 10 or more sugars. Various techniques are used for the structural analysis of oligosaccharides. Hu D. *et. al.* in 2020 study to use Ultra-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass/Mass Spectrometry (UPLC-QTOF MS/MS) for the identification of oligosaccharides (trisaccharides containing three monosaccharides (DP3) and oligosaccharides containing seven monosaccharides (DP7), including two pairs of isomeric oligosaccharides and three branched oligosaccharides) and Ultra-high Performance Liquid Chromatography With Charged Aerosol Detector (UPLC-CAD) for their quantification in different samples of *P. Heterophylla* [10].

While methods such as Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) and Nuclear magnetic resonance (NMR) are preferred for structural analysis, column chromatography and/or High-performance liquid chromatography (HPLC) techniques are widely used in the isolation of oligosaccharides, where carbon-celite is used as sorbent [1]. Instead of high-cost and operating cost devices such as MALDI-TOF and NMR, techniques such as capillary electrophoresis with Ultraviolet light-emitting diode (UV-diode) array detectors, high-performance anion exchange chromatography with amperometric detectors, high-performance liquid chromatography with refractive index detectors, thin layer chromatography, and UV-spectrophotometer combined with artificial neural networks are used for the quantitative determination of oligosaccharides in a product. [1]. It is also reported that xylooligosaccharides can be identified more quickly and reliably with hydrophilic interaction chromatography column - evaporative light scattering detection (HILIC-ELSD). Qualitative and quantitative structural analyses of oligosaccharides were also performed with electrospray ionization-mass spectrometry [1]. Another study determined the concentrations of 14 neutral oligosaccharides and 6 acidic oligosaccharides, as well as N-acetylneuraminic acid, using the internal standards stachyose and galacturonic acid, respectively. In the study, the crude milk oligosaccharide fraction underwent a separation process that involved the use of gel permeation chromatography, a technique that facilitates the isolation of various oligosaccharide fractions. This separation method yielded three distinct categories of oligosaccharides: acidic oligosaccharides, neutral oligosaccharides, and lactose. Subsequent to the separation stage, the neutral and acidic oligosaccharides were analysed in isolation through the utilisation

## Oligosaccharide content in cream formulation of *Triticum vulgare* L. extract

of a high-pH anion-exchange chromatography with pulsed amperometric detection method. [11] High performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD), a widely utilised method for carbohydrate analysis, provides limited chemical information on the detected peaks. Coulier et al., in order to enhance the detection and broaden the chemical information of the carbohydrates, HPAEC was coupled with mass spectrometry (MS). Utilising a pooled hydrolysate sample, it was demonstrated that HPAEC–MS possesses the capability to separate and detect numerous oligosaccharides within a single experimental run, with the basis of separation being retention time and mass. [12]

In the present study, we report a simple extraction method and efficient high performance anion exchange chromatography method reported for determination of oligosaccharides in cream form for *Triticum vulgare* L. extracts.

## 2. Experimental

### 2.1 Chemicals

*Triticum vulgare* L. aqueous extract (6 g/L, Farmaceutici Damor), sodium hydroxide (98-100 %, Sigma-Aldrich), sodium acetate trihydrate (Extra pure, Isolab Chemicals)

### 2.2. Extraction

No extraction process was performed in Abdi Ibrahim Laboratory for this study. The raw material *Triticum vulgare* L. aqueous extract is supplied from Damor supplier.

### 2.3. Chromatographic Conditions and Equipment

Ion Chromatography, Metrohm, 19401540, Herisau/Switzerland, the analytical method for separation and quantitation of total oligosaccharides was developed on Methrom, Ion Chromatography. The chromatographic instrument included a quaternary solvent pump, an auto-sampler, and an Amperometric Detector. The chromatographic conditions, such as the selection of solvents for solution preparation, the selection of the stationary phase for separation, the selection of the mobile phase for elution and the selection of the detection wavelength, were based on the solubility and physicochemical properties of the standards. Thermo Dionex Carbopac PA1 Guard, 4.0 x 50 mm, Thermo Dionex Carbopac PA1 4.0 x 250 mm was selected as a stationary phase.

Gradient elution technique was selected for the analytical separation of oligosaccharides from the matrices using Ion chromatography method by using a gradient of Mobile Phase A (Sodium Hydroxide) and Mobile Phase B (Sodium Acetate Trihydrate-Sodium Hydroxide) (Table 1). The flow rate of the mobile phase was adjusted to 0.5 mL/min with 1000 µL injection volume. The column oven temperature was fixed at 35 °C and sample temperature were used as 25 °C. The run time of the method was set to 85 min.

Note: It was observed that the electrodes in the device used during the analysis showed rapid contamination during mobile phase transitions and the retention times of the active substance in our analysis varied within the injections. In order to prevent this situation from being repeated in other parameters, the analysis process was started after cleaning the electrode with 2 Molar nitric acid solution.

#### 2.4.1. Preparation of Mobile Phase A

2.0 g sodium hydroxide is weighed into a 1000 mL flask. A certain amount of deionized water is added. It is dissolved. Its volume is made up with deionized water.

### 2.4.2. Preparation of Mobile Phase B

8.203 g of sodium acetate trihydrate is weighed into a 1000 mL flask. After dissolving, 2.0 g of sodium hydroxide is weighed on it. The volume is made up with deionized water.

**Table 1.** Gradient program of applied method

| Time (min) | Mobile Phase A | Mobile Phase B |
|------------|----------------|----------------|
| 0.0        | 95             | 5              |
| 5.0        | 95             | 5              |
| 15.0       | 85             | 15             |
| 30.0       | 85             | 15             |
| 30.1       | 80             | 20             |
| 40.1       | 80             | 20             |
| 40.2       | 70             | 30             |
| 50.2       | 70             | 30             |
| 50.3       | 65             | 35             |
| 60.3       | 65             | 35             |
| 75.0       | 0              | 100            |
| 76.0       | 95             | 5              |
| 85.0       | 95             | 5              |

**Table 2.** Detector Program

| Time (second) | E (Volt)                |
|---------------|-------------------------|
| 0.00          | +0.05                   |
| 0.20          | +0.05 (Integration ON)  |
| 0.40          | +0.05 (Integration OFF) |
| 0.41          | +0.75                   |
| 0.60          | +0.75                   |
| 0.61          | -0.15                   |
| 1.00          | -0.15                   |

### 2.4.3. Solution Preparation

For the stock standard solution, 7 mL of *Triticum vulgare* L. standard is transferred to a 150 mL volumetric flask. The volume is completed with deionized water.

For the preparation of Standard Solution 1; 2.5 mL of the Stock Standard Solution is transferred to a 20 mL volumetric flask. The volume is made up with deionized water. For the preparation of standard solution 2; 3 mL of the stock standard solution is transferred to a 20 mL volumetric flask. The volume is made up with deionized water. For the preparation of standard solution 3; 4 mL of the stock standard solution is transferred to a 20 mL volumetric flask. The volume is made up with deionized water. For standard solution 4; 5 mL of the stock standard solution is transferred to a 20 mL volumetric flask and the volume is made up with deionized water. For standard solution 5; 6 mL of the stock standard solution is transferred to a 20 mL volumetric flask and the volume is made up with deionized water.

### 2.5. Method Validation

The ion chromatography assay method for the finished product is explained in the Abdi Ibrahim in-house method and used ICH Q2 (R1) guideline for analytical validation parameters [13]. Equipment, columns, standards, etc. used in validation is stated in the protocol. Method validation parameters were

## Oligosaccharide content in cream formulation of *Triticum vulgare* L. extract

selected as specificity, accuracy, linearity, precision. Limit of Quantitation (LOQ) and Limit of Detection (LOD) studies were not performed since the study was an assay validation. LOQ and LOD studies were not performed as they are not required in the table under the heading 'Types of Analytical Procedures to be Validated' in the ICH Q2 (R1) guideline for assay validation. In accordance with the ICH Q2(R1) guideline, LOD and LOQ evaluations will be considered in subsequent validation phases or when required by regulatory submissions. The absence of these parameters in the current phase does not compromise the method's suitability for its intended purpose.

### 2.5.1. Specificity

Specificity is the observation of only analyte peaks at the retention time of the target analyte in the presence of other components (impurities, matrix components and degradation products). The target analyte is precisely measured and accurately identified in the matrix without any interference. It was used to determine the ability to measure only the substance intended to be measured in the sample analyzed. The specificity of the developed Anion-Exchange Chromatography-Amperometric Detector (AEC-AD) method was determined by direct analysis of the entire prepared standard solutions, *Triticum vulgare* L. extract and sample. The AEC-AD method was preferred in order to achieve the required selectivity and sensitivity in the matrix and to eliminate the negative effects of the interventions.

### 2.5.2. Accuracy

Accuracy is a parameter that expresses the closeness or estimation of the measurement results to the real quantity. The % recovery value is one of the checked parameters for accuracy. This value was calculated based on AEC-AD data for each analyte according to the following formula (Eq. 1).

$$\text{Recovery \%} = \text{Standard concentration} / \text{injected concentration} \times 100 \quad (1)$$

### 2.5.3. Linearity

In this study, a calibration curve was used to quantify the solutions by AEC-AD. To this end, five replicate measurements were performed on solutions at various concentrations, and a calibration curve was obtained using the nominal values of the analytes

### 2.5.4 Precision and Repeatability

The results of the repeatability and precision studies of the analytical method confirm the precision of the developed analytical method. Repeatability studies are performed by injecting the sample solution of medium concentration level in six replicates on the same day (see in support.info. Table 5.). Intermediate precision is performed to calculate the degree of deviation between measurements obtained from repeated sampling of the same sample under certain conditions. A solution of Active Oligosaccharide at a concentration of 0.00224 mg/mL is prepared. The prepared solutions are administered in 6 injections of 85 minutes each. When samples were injected six times on the same day, the type of precision was intraday precision.

## 3. Results and Discussion

### 3.1. Method Validation

#### 3.1.1 Specificity

The peak purity of each analyte in the standard, placebo solution and sample solutions confirm that the developed method is specific for the analytes of interest only and their separation and quantification is not affected by the presence of other chemicals present in the solutions and solution

mixtures. An AD detector was used to check the purity and uniformity of all peaks of *Triticum vulgare* L. The test solution containing *Triticum vulgare* L. extracts and sample solutions was injected into the Ion-chromatography system at the optimized chromatographic conditions. The chromatogram for the assay solution is shown in Figure 1. In this study there is no peak from the solvent and placebo at the retention time of the *Triticum vulgare* L. peak. The selectivity of the method has been proven.

**Table 3.** Results for Specificity Injection (\*NA: Not Applicable)

| Solution Name       | Retention Time (minute) | Area (AU x minute) | Asymmetry Factor | Theoretical Plates | Capacity Factor |
|---------------------|-------------------------|--------------------|------------------|--------------------|-----------------|
| Placebo solution    | *NA                     | *NA                | *NA              | *NA                | *NA             |
| Standard-1 Solution | 41.043                  | 8047.4396          | 1.136            | 15877              | 33.203          |
| Standard-2 Solution | 41.060                  | 9728.4293          | 1.189            | 15723              | 33.217          |
| Standard-3 Solution | 40.710                  | 12652.4809         | 1.326            | 14185              | 32.925          |
| Standard-4 Solution | 40.110                  | 16181.3268         | 1.381            | 13518              | 32.425          |
| Standard-5 Solution | 39.460                  | 19834.0013         | 1.507            | 12678              | 31.884          |
| Sample Solution     | 37.594                  | 14618.5033         | 1.728            | 14067              | 30.328          |

### 3.1.2. Linearity and Range

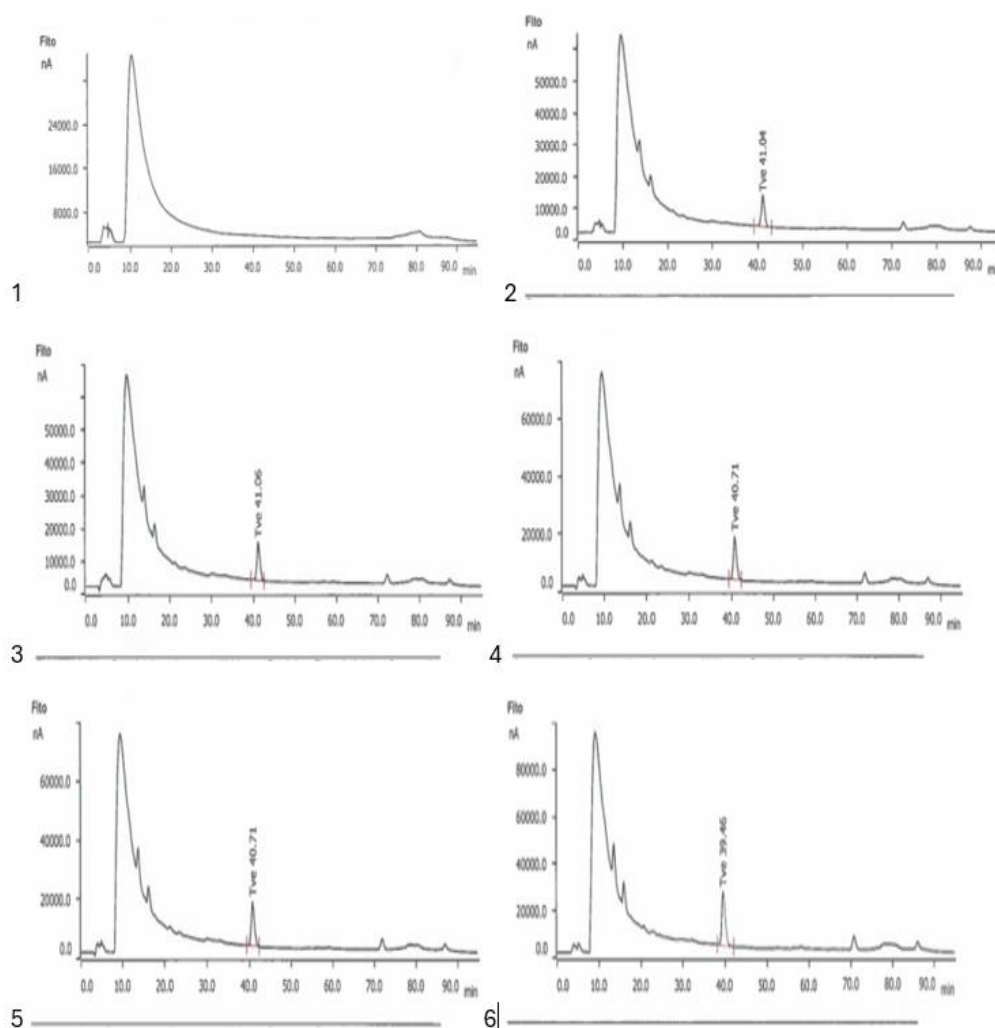
In order to prove the validated *Triticum vulgare* L. linear response relationship, 5 different concentrations of standard solutions were prepared. Concentration / area plots were drawn according to the linear regression method. Correlation coefficient (r) and regression were calculated on this graph (Figure S1).

The linearity studies were performed to confirm that within the selected range of *Triticum vulgare* L. (0.00140 mg/mL to 0.00336 mg/mL) the responses obtained are in direct proportion to the concentration. The linear relation was confirmed with the plot of peak area vs. concentration. Figure 3 represents the overlay linearity graph for *Triticum vulgare* L. in the selected concentration range. The linearity equation is included in Table 4. For both analytes, the regression coefficient value is greater than 0.9, which confirms that the developed method is linear in the selected concentration range of analytes.

**Table 4.** Summary of Linearity

| Solution Name   | Concentration (mg/mL)     | Area       |
|-----------------|---------------------------|------------|
| Level 1         | 0.00140                   | 8090.4986  |
| Level 2         | 0.00168                   | 9806.4534  |
| Level 3         | 0.00224                   | 12746.6615 |
| Level 4         | 0.00280                   | 16471.7450 |
| Level 5         | 0.00336                   | 19927.0579 |
| Linear Equation | $Y = 6204.20x - 737.0873$ | -          |
| R               | 0.999                     | -          |

### Oligosaccharide content in cream formulation of *Triticum vulgare* L. extract



**Figure 1.** Chromatogram sample blank solution, 2<sup>nd</sup> chromatogram sample standard-1 solution (0.0014 mg/mL), 3<sup>rd</sup> chromatogram sample standard-2 solution (0.00168mg/mL), 4<sup>th</sup> chromatogram sample standard-3 solution (0.00224 mg/mL), 5<sup>th</sup> chromatogram sample standard-4 solution (0.0028 mg/mL), 6<sup>th</sup> chromatogram sample standard-5 solution (0.00336 mg/mL)

#### 3.1.3. Accuracy

The suitability of the analytical method for the determination of samples in the matrix is confirmed by the accuracy studies to a great extent. The study confirms the optimum extraction of the analytes from the sample matrix. The accuracy of the method is demonstrated for 63% Level 1(0.0014 mg/ml), 100% Level 2 (0.00224mg/ml), 150% Level 3 (0.00336 mg/ml) by adding *Triticum vulgare* L. standards over placebo and calculating % recovery for each analyte, at each level. The recovery data for the present method are presented in Table 5. The % recovery for both analytes were in the Ich acceptable range of 85 - 115 %.

**Table 5.** Results of Accuracy (n=9)

| Name                          | Recovery (%) |
|-------------------------------|--------------|
| Level 1-1                     | 103.2        |
| Level 1-2                     | 105.1        |
| Level 1-3                     | 102.2        |
| Level 2-1                     | 113.4        |
| Level 2-2                     | 104.2        |
| Level 2-3                     | 108.0        |
| Level 3-1                     | 97.4         |
| Level 3-2                     | 99.1         |
| Level 3-3                     | 98.2         |
| Average                       | 103.4        |
| Standard Deviation            | 5.1          |
| % RSD                         | 4.9          |
| Confidence Interval<br>(95 %) | 103.4 ± 3.6  |

### 3.1.4. Precision and Repeatability

The results of repeatability and precision studies of the analytical method confirm the precision of the developed analytical method. The repeatability studies are performed by injecting sample solution at a medium concentration level in six replicates on the same day. 0.00224 mg/mL concentration of Active Oligosaccharide solution was prepared. Each of the prepared solutions was administered in 6 injections of 85 minutes each. When the samples are injected six times on the same day, the type of precision is intraday precision. For each sample, the mean peak area and % Relative Standard Deviation (%RSD) were calculated. %RSD below 15 confirms that the developed method is precise within the selected concentration range, as per ICH guidelines (Table S1).

## 4. Conclusions

The Selectivity, Linearity, Precision, Accuracy and Recovery study with the sample used in the active study of *Triticum vulgare* L. Extract was successfully completed. In the selectivity study, there was no interference between the prepared solutions. In the linearity study, we determined that it increased linearly by giving consistent and accurate results at 5 different concentration levels. In the accuracy study, we determined that the device and the prepared standard solution worked stably in the system accuracy parameter. In the repeatability parameter, we determined that the samples prepared under the same conditions and the device gave stable, accurate results. In the accuracy and recovery study, we determined how much of the standards added to the placebo samples prepared in the accuracy and recovery study were able to measure accurately during the analysis process.

In this study, the previous ion chromatograph was not able to maintain data integrity and was replaced by a newer instrument because it was an older instrument. The device used in this study is more modern and can be operated in accordance with data integrity rules. When the validation studies between the previous device and the new device are compared, it is seen that the validation results were within the acceptance criteria and the results were similar. While the results of 6 consecutive replicates of the previous device were 1.06%, 1.05%, 1.03%, 1.03%, 1.04 %, 1.08 %; the results obtained in the validation results of the new device were 1.10%, 1.07%, 1.07%, 1.06%, 1.07%, 1.08%. The %RSD of the old analysis results was 1.9%, while the %RSD result in this study was 1.3%.

## Conflicts of Interest

Authors do not have any conflicts of interest to declare.

## Acknowledgement

We would like to thank Abdi Ibrahim Pharmaceuticals for their valuable contribution to this study through laboratory and analytical support.

## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/journal-of-chemical-metrology>

ORCID 

Yeter Arten: [0009-0001-7374-0291](https://orcid.org/0009-0001-7374-0291)

Şafak Şanlı: [0009-0008-4129-5171](https://orcid.org/0009-0008-4129-5171)

Nurhan Metan: [0009-0006-9984-6509](https://orcid.org/0009-0006-9984-6509)

## References

- [1] L. Sanguigno, A. Casamassa, N. Funel, M. Minale, R. Riccio, S. Riccio, F. Boscia, P. Brancaccio, L. E. Pollina, S. Anzilotti, G. Di Renzo and O. Cuomo (2018). *Triticum vulgare* L. extract exerts an anti-inflammatory action in two in vitro models of inflammation in microglial cells, *PlosOne* **13(6)**, e0197493.
- [2] M. Demirci, O. Sağdıç, M. Çavuş, H. Pehlivanoğlu, M. T. Yılmaz and M. Çağlar (2017). Prebiyotik oligosakkaritlerin kaynakları, üretimleri ve gıda uygulamaları, *Avrupa Bilim ve Teknol. Derg.* **6(10)**, 20-31.
- [3] CosmeticsInfo. (2024). *Triticum vulgare* L. (Wheat Germ) Oil. Cosmetics Info.
- [4] R. G. Crittenden and M. J. Playne (1996). Production, properties and applications of food-grade oligosaccharides, *Trends Food Sci. Technol.* **71**, 353–361.
- [5] S. G. Prapulla, V. Subhaprada and N. G. Karanth (2000). Microbial production of oligosaccharides: a review, *Adv Appl Microbiol.* **47**, 299–343.
- [6] Q. Xu, Y. Chao and Q. Wan (2009). Health benefit application of functional oligosaccharides, *Carbohydr. Polym.* **77(3)**, P435-441
- [7] S. Marionneau, A. Cailleau-Thomas, J. Rocher, B. Le Moullac-Vaidye, N. Ruvoën, M. Clément and J. Le Pendu (2001). ABH and Lewis histo-blood group antigens, a model for the meaning of oligosaccharide diversity in the face of a changing world, *Biochimie.* **83(7)**, 565-573.
- [8] C. Zhao, Y. Wu, X. Liu, B. Liu, H. Cao, H. Yu, S. D. Sarker, L. Nahar and J. Xiao (2017). Functional properties, structural studies and chemo-enzymatic synthesis of oligosaccharides, *Trends Food Sci. Technol.* **66**, 135-145.
- [9] B. H. Li and X. S. Ye (2020). Recent advances in glycan synthesis, *Curr. Opin. Chem. Biol.* **58**, 20-27,
- [10] D. Hu, B. Han, C. Chen, N. Chen, B. Zhu, J. Zhao and S. Li (2020). Determination of seven oligosaccharides and sucrose in *Pseudostellaria heterophylla* by pressurized liquid extraction and ultra-high performance liquid chromatography with charged aerosol detector and tandem mass spectrometry, *J. of Chromatogr. A*, **1609**, 460441.
- [11] S. Thurl, B. Müller-Werner and G. Sawatzki (1996), Quantification of Individual Oligosaccharide Compounds from Human Milk Using High-pH Anion-Exchange Chromatography, *Analytical Biochemistry*, **235**, **2**, 202-206, 0003-2697.
- [12] L. Coulier, Y. Zha, R. Bas and P. J. Punt (2013), Analysis of oligosaccharides in lignocellulosic biomass hydrolysates by high-performance anion-exchange chromatography coupled with mass spectrometry (HPAEC–MS), *Bioresour. Technol.*, **133**, 221-231, 0960-8524.
- [13] ICH harmonised tripartite guideline, (27 October 1994). Validation of analytical procedures: text and methodology Q2(R1), Current Step 4 version Parent Guideline.

**A C G**  
publications

© 2025 ACG Publications